Overwintering of the Leafhopper *Graminella nigrifrons* (Homoptera: Cicadellidae) in Northern Ohio[1]

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ABSTRACT. **_Graminella nigrifrons_ was collected from the field in the fall of 1981 and placed in field cages containing fescue sod. Sixteen newly emerged _G. nigrifrons_ were collected with a vacuum sampler the following spring. In the winter of 1982–1983, _G. nigrifrons_ overwintered poorly on orchardgrass, better on ryegrass, and best on fescue. This data suggests that _G. nigrifrons_ overwintered as eggs. Stadia of development for _G. nigrifrons_ were one week longer on fescue than on ryegrass. Compared to naturally infested caged areas, those into which an additional 2,636 field-collected leafhoppers were released resulted in a 2.3-fold increase in leafhopper survival. The vacuum sampler collected more leafhoppers than did small (130 cm^2), yellow, adhesive-coated cards. Yellow cards captured more males than females, whereas the vacuum sampler collected more females than males inside the cages. The increased numbers of _G. nigrifrons_ in the caged area after the fall release, and the 1:1 sex ratio found on sod areas outside cages are evidence that _G. nigrifrons_ overwinters in northern Ohio. Fescue and ryegrass may serve as important overwintering hosts.**

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INTRODUCTION

The blackfaced leafhopper, *Graminella nigrifrons* (Forbes), is the most important vector of maize chlorotic dwarf virus (MCDV) (Nault and Knoke 1981), with both nymphs and adults transmitting MCDV in a semipersistent manner (Nault et al. 1973). Almost all maize lines (*Zea mays* L.) are susceptible to MCDV (Guthrie et al. 1982, Findley et al. 1983). Maize chlorotic dwarf is one of the two most widely distributed (Gordon and Nault 1977) and important (Gordon et al. 1981) virus diseases of maize in the United States. This disease is important in areas south of the corn belt in the eastern half of the United States (Gordon and Nault 1977, Nault et al. 1976). In this region, *G. nigrifrons* coexists with johnsongrass, [*Sorghum halepense* (L.) Pers.], the overwintering host of MCDV. In southern Ohio, MCD is epiphytotic on maize grown in river bottom land. The biology of *G. nigrifrons* has been studied under experimental conditions, and information on stadia of development, fecundity, and host range (Stoner and Gustin 1967) is available. However, information on the overwintering of *G. nigrifrons* in northern Ohio is lacking. DeLong (1971) stated that leafhoppers may overwinter as eggs or, in milder areas, as adults. In Mississippi, adult *G. nigrifrons* are commonly found throughout the winter on oats (*Avena sativa* L.), ryegrass (*Lolium perenne* L.), wheat (*Triticum aestivum* L.), and bahia grass (*Paspalum notatum* Flugge) (Pitre and Hepner 1967). *Graminella nigrifrons* comprised over 20% of the leafhopper species found on various small grains and grasses during the summer in northern Ohio (Knoke et al. 1983). Stoner and Gustin (1967) suggested that *G. nigrifrons* may overwinter either as eggs or adults in southern Ohio but presented no evidence. The present report provides evidence for the overwintering of *G. nigrifrons* in northern Ohio.

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MATERIALS AND METHODS

Two experiments were conducted to determine whether and how *G. nigrifrons* overwintered in northern Ohio. In the first, a permanent pasture area that supported large summer leafhopper populations (Knoke, unpublished data) near Wooster, OH was selected as a test site in 1981. This area was seeded with tall fescue (*Festuca arundinacea* Schreb. var. Kentucky 31) in August 1977. On 9 September 1981, six cage supports were positioned 91.4 cm apart, from north to south, over the sodded area. The sides of four U-shaped frames (each 1.8 m wide) of a cage support were taped together forming a cube. On the same day, bottomless cages constructed of 32 mesh saran screen (Chicopee, Peachtree Corners Plaza, Suite 1950, Atlanta, GA) were placed over the frames at positions one, three, four and six (one=south position, six=north position). Support frames in positions two and five were not covered with a cage. Each cage had a zipper in the center of the side facing east. The bottom edges of the cage were first secured to the ground using metal stakes inserted through grommets (located every 30.5 cm around the base of the cage). They were then covered with soil to prevent entry or exit of insects at the cage-soil interface. Three experimental conditions were: 1) laboratory-reared adult *G. nigrifrons* released into cages (position one and four); 2) field-collected insects (mostly leafhoppers) taken with a sweep net from the grassy area adjacent to the cages and released into cages (position three and six); and 3) unrestricted natural infestation (position two and five). Each condition was replicated twice.

Laboratory *G. nigrifrons* were reared on oats and maize in dacron-organdy-covered cages (38 cm x 38 cm x 38 cm) in a greenhouse-rearing room kept at 22-28° C and 50–70% RH, with 16 hr artificial light per day. After the adults emerged, they were released into a transfer chamber, counted, placed in an empty rearing cage for transport to the field site, and released inside the field cages. Batches of 500 leafhoppers per cage were released twice weekly, depending on weather conditions, on 18 dates between

14 September and 2 November.

Field-collected insects taken with a 40.6 cm diameter sweep net on 25 dates between 14 September and 4 November were released into cages three and six. The number of leafhoppers for each release was estimated by counting the insects collected in a 50-sweep sample. The leafhoppers were preserved in 70% alcohol until they were identified and counted. We estimated from the 50-sweep samples that a total of 3,804 *G. nigrifrons* adults was introduced into each cage.

The four cages were removed from the support frames on 25 November to permit snow cover on the grass. On 12 March 1982, cages were placed over all six frames. Beginning 17 May and at about weekly intervals until 21 June, a D-Vac suction sampler (D-Vac Company, P.O. Box 2095, Riverside, CA) was used to determine the leafhopper population in each cage. After entering the cage through the zipper door, the D-Vac's sampling head (929 cm^2) was operated just above the grass in the cage and over the inside surfaces of the top and sides of the cage. During this same period, leafhopper populations were also sampled from a nearby 3.3 m^2 area seeded to orchardgrass (*Dactylis glomerata* L.) and ryegrass. The insects caught were preserved in 70% alcohol until the leafhoppers were identified and counted.

A second overwintering study was conducted during the winter of 1982–83 on pure grass strands, and it involved sampling leafhopper populations inside and outside the caged areas with yellow adhesive-coated cards as well as with the D-Vac suction sampler. Originally, five varieties of grass were hand-planted in the field on 15 September 1981 as three replicates in a randomized complete block design consisting of a 4.6 m x 7.6 m area per host per replicate. A 4.6 and 3.1 m unseeded area separated the grasses and replicates, respectively. However, the winter survival of *G. nigrifrons* was only determined on three of the five surviving grasses (ryegrass, tall fescue, and orchardgrass). Two support frames per grass area were positioned so that the frames were 1.3 m apart and equidistant from the plot ends. About 1.4 m of grass area remained at the sides of each frame. The east cage support in each plot was covered with a saran screen cage from 16 September to 30 November. A total of 1,240 male and 1,396 female field-collected *G. nigrifrons* that were collected from a nearby sodded area was introduced into each cage on 14 dates between 20 September and 8 November 1982. As in the 1981–82 study, the screen cages were removed for the winter. In two adjacent plots, the number of leafhoppers on three replicates of noncaged areas of Kentucky bluegrass (*Poa pratensis* L.) and timothy (*Phleum pratense* L.) was also determined.

Cages were re-positioned on the frames from 3 March to 7 July to determine whether any leafhoppers overwintered on the grasses. Sampling with the D-Vac suction sampler within the cage was done as previously described. Sampling outside the caged areas and the noncaged areas of Kentucky bluegrass and timothy plots was done while holding the sampling head just above the grass surface and walking once around the edge of the plot (22.9 linear meters per plot). Leafhoppers were also sampled with 10.2 cm by 12.7 cm yellow cards that were coated with adhesive on both sides (Olson Products, Inc., P.O. Box 1043, Medina, OH) and held just above the grass surface on wire supports. The adhesive cards were positioned in the center of each seeded area (between the cages) and two feet from the zipper door within each cage or in the center of the noncaged areas of the Kentucky bluegrass and timothy plots.

RESULTS AND DISCUSSION

Detection of winter survival of *G. nigrifrons* in northern Ohio in mid–1982 was difficult. Few *G. nigrifrons* were collected by vacuum sampling inside the six cages during May and June, 1982. Four adults were obtained from the two cages where 18,000 laboratory-reared adults were previously released and resulted in a fall-to-spring ratio of 0.02 per 100 introduced adults. The cages into which field-collected leafhoppers were released yielded three adults (0.04 per 100 introduced adults), while the areas naturally infested (noncaged) in the fall produced nine *G. nigrifrons*. During this same period, a total of 50 adult *G. nigrifrons* was collected by vacuum sampling 3.3 m^2 of a nearby area seeded to orchardgrass and ryegrass. These naturally occurring leafhoppers may have resulted from eggs laid in the fall on these grasses, from local migrants of other nearby grassy areas, or from long distant migrants. Their nontattered physical condition suggested that they were newly emerged adults of local origin (Stoner and Gustin 1967). All adult leafhoppers collected inside and outside the cages also appeared to be newly emerged adults, suggesting that overwintering occurred in the egg stage on local grasses.

The 1982–1983 winter data from caged and noncaged areas of different grass hosts strongly support the notion that *G. nigrifrons* overwintered in northern Ohio (Table 1). For each of the three grasses, cages with released field-collected *G. nigrifrons* in the fall yielded about twice as many adults as did cages infested naturally. Based on the total number of adult *G. nigrifrons* collected by both methods, the fall-to-spring ratios (number of newly emerged adults collected in the spring per 100 introduced adults) were 1.93, 3.30, and 0.61 on ryegrass, fescue, and orchardgrass, respectively. These ratios were considerably higher than the 0.04 ratio on fescue the previous winter. Stadia of development, as indicated by the number of adults caught, was faster on ryegrass than on fescue and orchardgrass. As in a previous study (Knoke et al. 1983), leafhopper collections from fescue and ryegrass were nearly equal and greater than collections on timothy. June-July leafhopper populations outside the cages were higher on ryegrass and fescue than on orchardgrass and timothy; populations on bluegrass were intermediate. The 68 adults collected from naturally infested caged areas and the 2.3-fold increase when leafhoppers were released into similar caged areas are evidence for winter survival of *G. nigrifrons* on these grasses.

The vacuum sampler collected more leafhoppers than the yellow, adhesive-covered cards. A 60-sec operation of the vacuum sampler collected an average of 4.8 times as many *G. nigrifrons* as the continuous one week exposure of adhesive-coated cards. It also collected more (56%) females than males inside the cages. Yellow cards captured (68%)

TABLE 1

Number of Graminella nigrifrons *(GN) adults collected on various grass hosts near Wooster, OH in 1983.*

Host	Grass caged[1]	GN added[2]	Sample method and collection date[3]							
			Vacuum sampler				Yellow adhesive card			
			21 Jun	27 Jun	5 Jul	Total	21 Jun	27 Jun	5 Jul	Total
Ryegrass	Yes	Yes	2	24	9	35	5	4	7	16
	Yes	No	5	7	5	17	7	1	1	9
	No	–	9	38	11	58	3	2	0	5
Tall fescue	Yes	Yes	0	0	76	76	1	0	10	11
	Yes	No	0	1	30	31	0	0	3	3
	No	–	11	17	25	53	1	8	2	11
Orchardgrass	Yes	Yes	1	3	6	10	0	0	6	6
	Yes	No	0	3	4	7	0	1	0	1
	No	–	1	3	3	7	0	1	3	4
Kentucky bluegrass	No	–	8	14	12	34	1	4	0	5
Timothy	No	–	6	8	8	22	0	0	2	2

[1]Grass area covered with a 32 mesh screen cage (1.8 m x 1.8 m x 1.8 m) from 16 September to 30 November 1982; both caged areas covered with cages from 3 March to 7 July 1983.

[2]An estimated total of 1,240 male and 1,396 female field-collected GN was released in each cage infested on 14 dates between 29 September and 8 November 1982.

[3]Total number of adults collected from three replicates on month/day.

more male than female *G. nigrifrons* at all sites, possibly because of the excitable behavior in males. Overall, there was a 1:1 male to female ratio in the 498 adults collected in the 1982-1983 study. This 1:1 sex ratio is additional evidence that the sampled individuals were of local origin as suggested by Drake and Chapman (1965).

The grass host at each site influenced the total number and sex composition of *G. nigrifrons* collected (Table 2). Included in the total numbers collected were the four *G. nigrifrons*-like nymphs and one adult female collected from inside the cages on 13 June 1983. In comparisons of winter survival between cages with released leafhoppers and cages without released leafhoppers, greater numbers of females were collected from cages over ryegrass and fescue with released leafhoppers. Similarly, greater numbers of males were collected from cages over fescue with released leafhoppers. However, the trend was not observed for males in cages over ryegrass and orchardgrass. Significant differences in numbers of insects collected between cages with and without released leafhoppers indicate that the host grass species supported winter survival. Further, differences within cage treatments on different grasses indicate the relative suitability of the grasses in supporting overwintering populations. Winter survival was best on fescue (64% of the collected leafhoppers), fair on ryegrass (25% of the total), and poor on orchardgrass (11% of the total).

The detection of nymphs in cages before adults were present, and the appearance of newly emerged (nontattered) adults in screened cages which excluded the possibility of migrants in these collections suggest that these individuals had overwintered as eggs. The exact site of oviposition within the cages was unknown. However, the different levels of leafhopper survival in cages positioned over the different host plants suggest that oviposition occurred on, within, or very near the host plants. Although these perennial hosts provided a means of overwintering for *G. nigrifrons*, they are not susceptible to MCDV (Nault et al. 1976) and, therefore, cannot serve as a virus source. Previous reports suggested that the presence of early season adults may be evidence for adult overwintering (DeLong 1971, Stoner and Gustin 1967) or spring migration (Chiykowski and Chapman 1965, Medler 1957). However, we saw no evidence of early season adults of *G. nigrifrons*.

Knowledge that *G. nigrifrons* can overwinter locally in northern Ohio and that the winter survival is host-related indicates that local populations may be modified by cultivation of appropriate host plants. Experimental increases in the populations of *G. nigrifrons* in areas beyond the normal range of MCDV may be desirable for evaluation of vector and virus resistance in maize. Higher vector populations may also be useful to study vector and virus movement. In contrast, altering host plants to decrease vector populations in areas where the disease is epiphytotic could reduce spring and summer populations and provide a measure of disease control. Further, knowledge of favorable overwintering hosts may suggest other measures for control of vectors.

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TABLE 2

Number of female and male Graminella nigrifrons *(GN) collected on various grass hosts near Wooster, OH in 1983.*

Host	Grass caged	GN added	Mean number GN per sample area[1]		
			Female	Male	Both[2]
Ryegrass	Yes	Yes	10.7 b	7.7 bcd	18.3 bc
	Yes	No	3.0 def	5.7 cde	9.0 cd
	No	–	11.0 bc	10.3 bc	22.0 b
Tall fescue	Yes	Yes	20.7 a	20.0 a	47.0 a
	Yes	No	12.7 b	8.7 bc	23.0 b
	No	–	7.3 bcd	14.0 ab	22.3 b
Orchardgrass	Yes	Yes	4.3 cdef	2.3 de	8.3 cd
	Yes	No	1.7 f	1.3 e	3.7 d
	No	–	2.7 def	1.0 e	3.7 d
Kentucky bluegrass	No	–	7.3 bcde	5.7 cd	13.7 bcd
Timothy	No	–	3.0 ef	5.0 cde	8.3 cd

[1]Number based on three replicates of four (yellow cards) and five (vacuum) sample dates. Means in the same column followed by the same letter are not significantly different ($P \geq 0.05$; Duncan's multiple range test).

[2]Also includes leafhoppers caught on 13 June 1983.

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